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# RELATIONSHIP BETWEEN SUBSTRATES, RESPIRATION AND STRUCTURE OF MITOCHONDRIA IN EUGLENA GRACILIS (Z)

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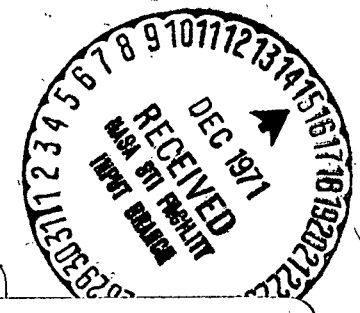




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# RELATIONSHIP BETWEEN SUBSTRATES, RESPIRATION AND STRUCTURE OF MITOCHONDRIA IN EUGLENA GRACILIS (Z)

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ABSTRACT. Euglena gracilis, strain Z, was grown in synchronous culture. Carbon source used was either DL-lactic acid (L) or a mixture of L-glutamic and DL-malic acids (GM). Synchronisation was obtained by transfering the cells in the exponential growth phase, at regular intervals - each 3 days - to a fresh medium. Respiration (measured during a whole cell cycle, 12 h) was 20 $\pm$6 $\mu$l/H/$10^5$ cells on (GM) and 46 $\pm$7 $\mu$l/H/$10^6$ cells on (L) medium. At the same time as the increased rate of oxygen uptake on lactate medium, we observed the appearance of a "giant" chondriome in the cells. On glutamate - malate containing medium the size of mitochondria was "normal".

Euglena gracilis (Z) is capable of using as source of energy and carbon for /308* its growth in darkness, different carbureted substrates which are generally intermediate compounds of the Krebs cycle (Hutner and Provasoli, 1951, 1955; Danforth 1953). Acitate and ethanol are also favorable substrates (Hutner and Provasoli, 1951, 1955; Cramer and Myers, 1952; Danforth and Wilson, 1957; Wilson and Danforth, 1958) as well as glucose (Cramer and Myers, 1952; Pringsheim, 1955; Hutner, Bach and Ross, 1956; Belsky, 1957; Danforth, 1962; Hurlbert and Rittenberg, 1962).

We shall demonstrate that lactate can make up an excellent carbon source for the Euglena. It is known that, in the case of the Euglena, the environmental conditions (light, darkness, carbon sources) can induce large-scale modifications of the chondrione (Lefort, 1964) as well as of the respiration rate (Sharpless and Butow, 1970a). During this work we have sought to define those carbureted substrates capable of playing a role as an inducer of these modifications simultaneously affecting the respiratory rate and the chondriome.

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[2]The electronic microphotos were made by Mr. C. Mattei, CNRS engineer.

*Numbers in the margin indicate pagination in the foreign text.

## Methods and Material



Euglena gracilis, strain Z (Eg) (Cambridge Alga Library no. 1224-5d) was cultivated in darkness at 27° C in an inorganic medium to which had been added as a carbon source either L-glutamic acid (14 mM) and DL-malic acid (15 mM) (GM) (Greenblatt and Schiff, 1959) or DL-lactic acid, 33 mM (L) (Calvayrac and Douce, 1970) (Merck).

The pH of the cultural medium plays an important role in the assimilation of carbureted substances by the Euglena (Danforth, 1953; Wilson, et al., 1959). In order to limit the number of parameters controlling the respiration, the cultural mediums used in this study were all at pH 3.5 and therefore only differ by the nature of the carbureted source.

The Euglenas were transferred to a new cultural medium every three days. The number of Euglenas in the culture was initially about 20 per µl. Under these conditions, after several transfers, at the same time following the increase by counting by means of the Malassey cell, we observed that the Euglenas became divided synchronously during at least 6 generations.

The respiration was measured by the polarographic method based on the Clark et al. electrode principle (1953) (membrane electrode) and is expressed in µl of oxygen consumed per hour for $10^6$ Euglenas ($\mu l O_2/H/10^6 Eg$).

In order to carry out the observation with the electron microscope, the Euglenas were removed in the exponential growth phase when the medium contained 400 to 600 Euglenas per µl, and in the 4 hours following their division. A prefixation of the material was carried out by means of a solution of 2% glutoraldehyde placed in the cultural medium for 15 minutes, followed by a post-fixation by a solution with 2% osmium tetroxide in the Palode buffer solution for 15 minutes. The sections were made with an LKB ultramicrotome and observations with an electron Hitachi "HS8" microscope.

## Results

Subjected to our cultural conditions as previously defined, the Euglenas have a generation time close to 12 hours (Figure 1) which allowed us to study the respiration during one complete cellular cycle (Figures 2 and 3).

The endogenous respiration of the Euglenas washed and placed in an inorganic medium was 20 $\pm$5 µl $O_2$/H/$10^6$Eg.

According to Figure 3 we note that in the (GM) medium the respiration was practically constant during one cellular cycle. It was in the vicinity of 20 $\pm$6 µl $O_2$/H/$10^6$Eg, which is on the same order of magnitude as the endogenous respiration.

In the (L) medium the respiratory rate was higher since it reached 46 $\pm$7 µl $O_2$/H/$10^6$Eg and represents more than double the endogenous respiration. Moreover, just before cellular division, the respiratory rate appeared to rise slightly.

Observation of the micrographs showed that the Euglenas cultivated in a (GM) medium had mitochondria whose size was not greater than two microns (Figure 4a). They were many in number and their matrix was filled with ridges grouped more or less in a cluster (Figure 4b).

The mitochondria of the Euglenas cultivated in the (L) medium measured up to /310 10 µ in length and often had a branched, fingerlike or anastomosed appearance (Figures 5a and b).

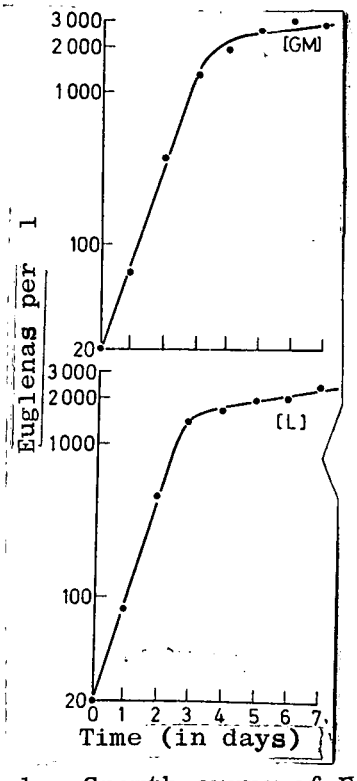



Figure 1. Growth curve of Euglena gracilis, strain Z, cultivated in darkness (27°C), in two media, one (GM) containing lactic acid. Generation time 12 hours.

## Discussion

The synchronization of the cellular division of the Euglenas in the two media (L and GM) studied allowed us to carry out cytological observations to a well determined physiological stage of their growth. We observe (Figure 3) that the quantity of /312 oxygen absorbed during the respiration of the Euglenas in the (GM) medium is approximately half of that consumed by Euglenas cultivated on the (L) medium. Thus, the results obtained with the glutamate-malate as carbon source are in the same order of magnitude as those obtained with glucose-supplemented media. When cultivated in a (GM) medium, the Euglenas have a respira-

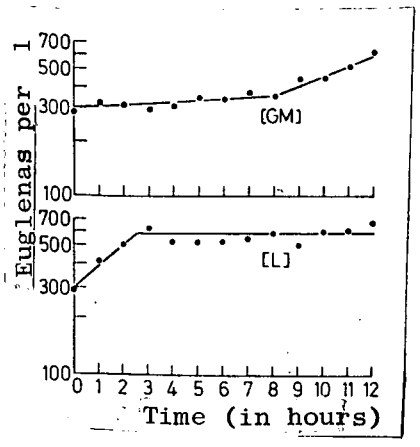



Figure 2. Synchronization curve of the division cycles of Euglena gracilis, strain Z, (27°C) in darkness, in 12 hours, cultivated in (GM) and (L) media and kept in an exponential growth phase by transferring every 3 days.

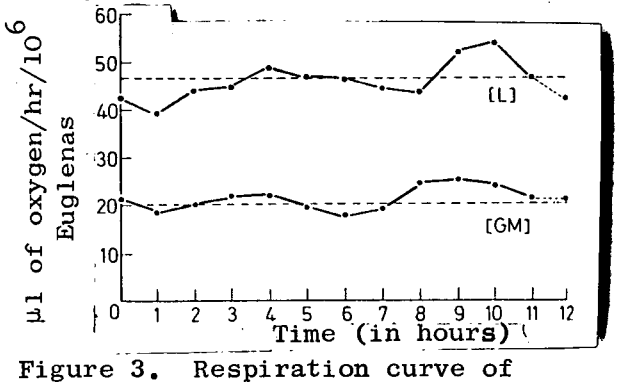
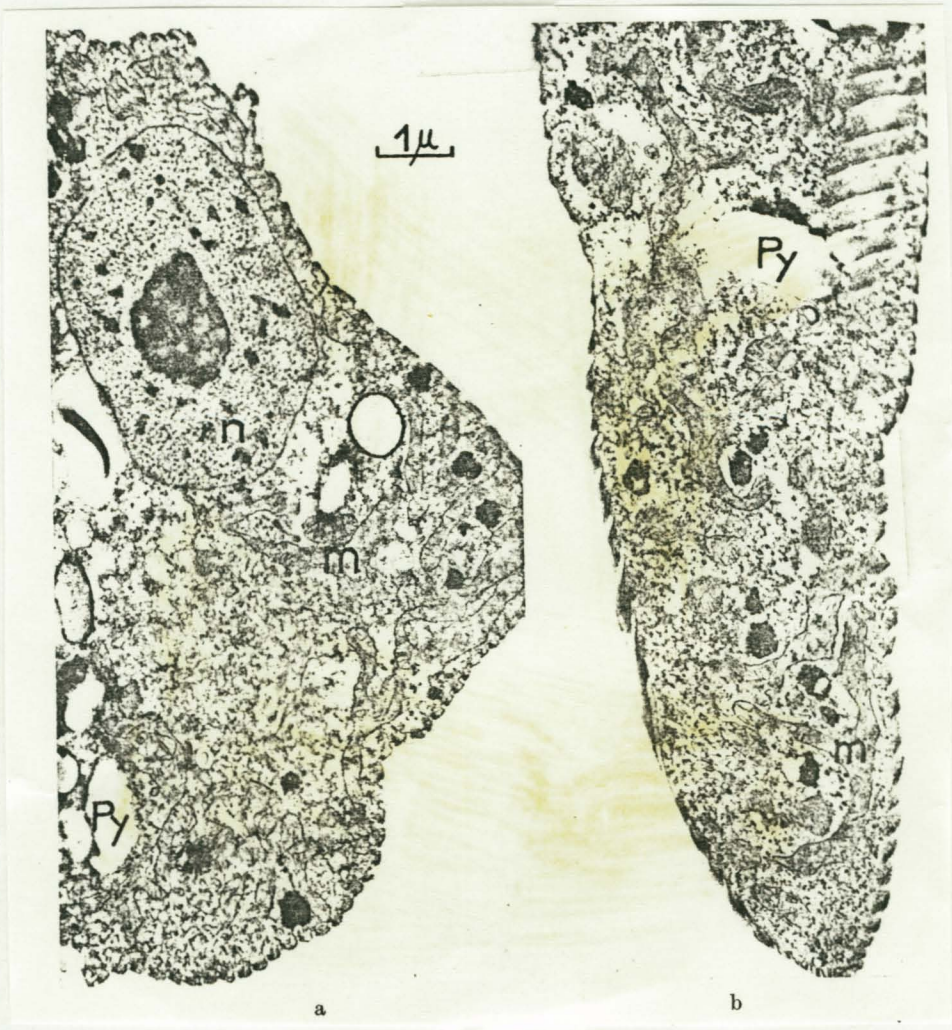



Figure 3. Respiration curve of Euglena gracilis, strain Z, during on division cycle in (GM) and (L) medium (27°C in carkness).

tion in the same order of magnitude as the endogenous respiration, or 20 µl $O_2$/H/$10^6$Eg (Boehler and Danforth, 1964; Cook and Heinrich, 1965; Heinrich and Cook, 1967) and /313 have, in this case, mitochondria of the conventional type (Walker, 1968; Buetow, 1968). On the contrary, the Euglenas cultivated in the (L) medium have an increased respiration, approximately the double of the endogenous respiration, as it has already been possible to show in the case of media supplemented with ethonol or acetate (Buetow, 1961; Heinrich and Cook, 1967). Under these conditions of culture, the Euglenas have mitochondria which are considerably changed. It is not yet possible to know whether the number and the mitochondrial surface increase correlatively or whether the appearance of broncheal mitochondria is induced by the coalescence of preexisting small mitochondria without increase in the membrane surface. It is only possible to ascertain, on one hand, a correlation between the appearance of this new chondriome and the increase in respiration and, on the other hand, the reversibility of the phenomenon since, at the time of the transfer in the (GM) medium, there is found in the Euglena the customary profile of the small mitochondria such as shown us in Figures 4a and 4b.

It has been independently demonstrated that the increase in respiration owing to the effect of certain carbureted metabolites such as ethonol, acetate and lactate is linked to the induction of a transporter chain of electrons insensitive to antimycine (Sharpless and Butow, 1970a and b).


Figures 4a and b. Euglena cultivated in (GM) medium in darkness. a - transverse section; m-mitochondria; n-nucleus; pp-proplast; Py-paramylon. b-sagittal section, the arrows show the cluster and grouping of the mitochondrial ridges.




Figures 5a and b. Euglenas cultivated in (L) medium in darkness. a-transverse section; m-mitochondria; n-nucleus; Py-paramylon. b-sagittal section.

Works presently underway should allow interrelating these three aspects of the same phenomenon.

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